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STUDY ON SEROPREVALENCE OF *MYCOBACTERIUM BOVIS* IN DIARY MILK FROM COW AND HERDSMEN'S SPUTUM IN KANO, NIGERIA

Abdul-Qadir, Z. A Department of Medical Microbiology and Parasitology, Bayero University, Kano, Nigeria

Mohammed, Y Department of Medical Microbiology and Parasitology, Bayero University, Kano, Nigeria

Rogo, L. D Department of Medical Laboratory Sciences, Bayero University, Kano, Nigeria.

Sani, M.N Department of Microbiology and Biotechnology, Federal University, Dutse Jigawa State -Nigeria

Abstract

Mycobacterium bovis is a chronic, contagious, debilitating, infectious disease affecting all age groups and of animals including humans. The prevalence for Mycobacterium bovis among herdsmen's sputum and diary milk of cattle in Bichi, Kumbotso, Rano, Rogo and Wudil local government areas of Kano State were investigated in this research 36 samples were collected from each local government expect Wudil which collect 38 samples of which, 91 from dairy milk of cattle and 91 from herdsmen's sputum. The ELISA M. bovis antigen kit was used to detect antigen against Mycobacterium bovis. Among 182 tested samples, 3 (1.65%) were positive against M. bovis antigen. All positive samples were from herdsmen's sputum while 0% was found in diary milk of cattle in studied areas.Although, a zero percent prevalence of bovine TB (bTB) was found in this research from raw cow milk,the isolation of M. bovis from sputum of herdsmen suggests potential of human to human infection especially in dense and over populated area. It also shows that bTB is of public health importance in Africa and beyond. The study also demands interventions since M. bovis infects both human and animal hosts. Hence, its control calls for an intersectional approach between the Medical, Veterinary professionals and Government.

Keywords: Bovine Tuberculosis, Diary milk, herdsmen's sputum, Kano

INTRODUCTION

Human *M. bovis* infection is one of the major public health risks and an important source of Tuberculosis (TB) to humans in 1930s as a result of high prevalence of bovine tuberculosis in cattle but the practice of test and slaughter programmer as well as introduction of pasteurization of milk reduced the incidence (Shitaye*et al.,* 2007). Currently, bovine tuberculosis is mainly endemic in developing countries including sub-Saharan Africa where both the test and slaughter policy have not been properly implemented (Cosivi*et al.,* 1998). Hence, bovine TB (BTB) is either partially controlled or not controlled at all which makes those working with cattle such as herdsmen, veterinarians, livestock workers to be at high risk of BTB infection (Georghiou*et al.,* 1989). *Mycobacterium bovis* can be transmitted to humans typically by the inhalation of aerosols, ingestion of unpasteurized milk, raw milk or eating under cooked beef can be a source of infection (Cousins and Dawson, 1999; Michel *et al*., 2010). The co-existence of farmers and the animals is exemplified by herdsmen, who live their entire lives with their animals, offering an ample opportunity for zoonotic transmission of infection. In most cases, *M. bovis*is transmitted through airborne infection among farmers, veterinarians and slaughters house workers between cattle in aerosols (Cousins and Dawson, 1999; Biet*et al*., 2005; Michel *et al*., 2010). It can also be through accidental laboratory exposure (OIE. 2009; Muller *et al.,* 2013). *M. bovis* is responsible for 5-10% of all human tuberculosis cases (Wedlock *et al.,* 2002) but infection rate usually varies from country to country (Muller *et al.,* 2013). Consumption raw milk is the primary route of *M. bovis* infection in humans (Miller *et al.,* 2013). After being exposed to infection, animals and humans respond by activating both cellular and humeral immunity. *M.bovis* moves via the lymphatic system within cells to regional lymph nodes just like other *Mycobacterial* infections and delayed hypersensitivity reactions commence between 30 to 50 days after infection has been established (Palmer and Waters, 2006; WHO, 2009; Good and Duignan, 2011), Tuberculosis is really a chronic infection. Many infected animals in the herd remain unnoticed for a very long time but they transmit the bacteria via milk, urine, feaces and by aerosol. Therefore, the infection spreads from chronically infected carrier animals to susceptible animals (Palmer and Waters, 2006; WHO, 2009).

Bovine tuberculosis remains a public health concern in developing countries including Nigeria. About 5-10% of all human tuberculosis cases are caused by *M. bovis*. Most human tuberculosis cases due to *M. bovis* occur in young individuals and result from drinking or handling contaminated milk, as a result, carnival lymphadenopathy, intestinal lesions, chronic skin tuberculosis (lupus vulgaris) and other non-pulmonary forms are common (Thoen*et al*., 2006). Information on human disease due to *M. bovis*across the globe is scarce and little is known in the study area. Tuberculosis is a major opportunistic infection in HIV infected persons, and infection with *M. bovis*compounds the disease, and in Nigeria there is high prevalence of people living with HIV and milk is a good source of nutritional value to all. It could be a serious public health threat to persons at risk (Moda*et al*., 1996, Dabor*et al*., 1997).

Mycobacterium bovis is transmitted between cattle to humans via aerosols during close contact, . These predisposing factors above are most predominant situations in Nigerian society. It is likely that *M. bovis* infection have been thriving unnoticed since report of epidemics is poorly documented in Nigeria. Therefore, there is need for a research of this type to provide necessary information for proactive strategy especially in the area of present study. .

MATERIALS AND METHODS

Study Area

The study was carried out in Kano State which is located in Northwest geopolitical zone of Nigeria. It comprises of 44 Local Government Area with an estimated population of 9,383,682 and 20,760 sq km in area (NPC, 2006). It lies between latitudes 10^{o} 33N to 11^{o} 15N and longitudes 34^{o}CE to 8^{o} 20CE (NPC, 2006). Cattle used for this research are sourced from individual owners in the different locations considered.

Study Population

This consisted of lactating cows and herdsmen.

Inclusion Criteria

All lactating cows and consented herdsmen were included.

Exclusion Criteria

All bulls, non-lactating cows and herdsmen that did give their consent..

Study Design

The Study is descriptive cross-sectional.

Determination of Sample Size

The number of samples used for the study was determined using sample size determination in health studies, (Lwanga and Lemeshow, 1991; Sarmukaddam and Grad, 2006) based on local prevalence study with 12.7% prevalence rate of *M.bovis* infection (Abubakar*et al.*, 2015).

$$N=\frac{Z^2\times P\,(1-P)}{d^2}$$

Where N= sample size-?
Z= standard normal distribution curve, (95% confidence level) =1.96
P= Prevalence rate from past studies-12.7%
d= absolute error – 5%

$$\frac{(1.96)^2 \times 0.127(1-0.127)}{(0.05)^2}$$

n=170.3

The sample size is approximated to182

Study Sampling Technique

The samples for the study were obtained using simple random sampling technique among the study population.

Ethical Consideration

An ethical approval was obtained from Kano State Ministries of Health in 4th May, 2017 and Agriculture in 9th June, 2017 with reference number NRS/128 before the commencement.

Sample Collection

Milk and sputum were collected in plain sterile container from cows and sterile sputum containers from herdsmen respectively in an aseptic manner and stored at -4°C on ice before taken to the laboratory, then stored at -20°C. The samples were used for ELISA analysis.

Serological Analysis for Detection of *Mycobacterium bovis* Antigen

Samples were screened for *M. bovis*antigen using commercially available *M. bovis*qualitative enzyme immune – assay determination kit (SUNLONG BIOTECH CO., LTD, 2017).

Principle of the Assay

The kit is based on sandwich enzyme-linked immune-sorbent assay technology. A 96 well plate has been pre-coated with an antibody specific to *M. bovis*. Standards or samples were added to the appropriate Microelisastripplate wells and combined to the specific antibody. Then a Horseradish peroxides (HRP) conjugated antibody specific for *M. bovis*is was added to each Micro ELISA strip plate well and incubated. Free components were washed away. The Tetramethylbenzidine (TMB) substrate solution was added to each well. Only the wells that contain bTB-ag and HRP conjugated bTB antibody appeared blue in colour and then turned yellow after the addition of stop solution. The optical density (OD) was measured spectrophotometrically at 450 nm. The presence of bTB antigen was determined by comparing with the CUTOFF value (SUNLONG BIOTECH CO., LTD, 2017).

Sample

Samples collected were immediately aliquot and store at -20°C.

Assay Procedure

The kit components and samples were equilibrated to room temperature prior to use. A standard curve for each test was plotted. In the Microelisa strip plate, two wells were left as negative control, two wells as positive control and one well empty as blank control.

Number: the sequential number, corresponding sample of the microporous hole 2 per board should set negative control and positive control 2 holes, ck 1 hole (ck hole without samples and HRP-Conjugate reagent, the rest of the same step operation). Adding samples: Negative and positive control in a volume of 50µl are added to the negative and positive controls wells respectively. In sample wells, 40ml sample dilution buffer and 10ml sample were added. Samples were loaded onto the bottom without touching the well wall. Mixed well and shake gently. Incubation: incubated for 30mins at 37^{o}C after sealed with Closure plate membrane.
Dilution: the concentrated washing buffer was diluted with distilled water (30mins for 96T).
Washing: the Closure plate membrane was carefully peeled off, aspirated and refilled with the washed solution. The wash solution was discarded after resting for 30secs. The washing procedure was repeated 5times. Fifty mile HRP-Conjugate reagent was added to each well except the blank control well. Incubation: it was incubated for 30mins at 37^{o}C after sealed with Closure plate membrane.
Washing: The Closure plate membrane aspirated and refilled with the wash solution. Wash solution was discarded after resting for 30secs. The washing procedure was repeated for 5times.Coloring: 50ml chromogen Solution A and 50ml Chromogen Solution B was added to each well, mixed with gentle shaking and incubated at 37^{o}C for 15mins in a light free environment. Termination: 50ml stop solution was added to each well to terminate the reaction. The color in the well changed from blue to yellow. Absorbance O.D. was read at 450nm using a Microtiter Plate Reader. The OD value of the blank control well was set as zero. Assay was carried out within 15 minutes after adding stop.

Results Interpretation

Test effectiveness: the average value of positive control ≥ 1.00; the average value of negative control ≤0.10The critical value (CUT OFF) calculation: critical value = the average value of negative control + 0.15Negative Result: if the OD value < CUT OFF, the sample is bTB antigen negative. Positive Result: if the OD value ≥ CUT OFF, the sample is bTB antigen positive.

Data Analysis

Data generated was analyzed using SPSS software version 20 (2011, IBM, Crop). The Prevalence of *Mycobacterium bovis* infection was expressed in simple proportions and percentages for the study group. Chi-square (X2) was used to determine the relationship between BTB infection and associated risk factors. A probability value of less than (0.05) was taken as significant.

RESULTS

Two groups were tested. Both groups consist of 91 samples. Ninety-one (91) raw milk samples and ninety-one (91) herdsmen sputum samples. Thirty six samples were collected per local government and 38 samples from Wudil making a total of 182 samples.

Samples	Negative (%)	Positive (%)	Total (%)
Diary milk	91 (100.0)	0 (0.0)	91 (100.0)
Herdsmen's sputum	88 (96.7)	3 (3.3)	91 (100.0)

The age group of herdsmen was between fifteen (15) to sixty (60). Age group of 35-54 was found to have positive samples 3.3% (Table 1). However, there is no significant relationship between the age group and *M. bovis* status of the subject at P value< 0.05.

Many risk factors for *M. bovis* transmission were examined such as exposure of residence to cattle herds, age group, ages of rearing the cattle by herdsmen, knowledge of *M. bovis* by herdsmen and symptoms.

The study shows that cows and milk samples were free from *M. bovis* antigen (Table 2) while 3.3% are herdsmen infected with *M. bovis*antigen (Table 2). These may be due to exposure of residence with cattle herds and the ages of rearing of cattle also play an important role in transmission of the disease (Table 3). The knowledge of *M. bovis* by the herdsmen plays an important role in contacting the disease (Table 4). The presence of clinical symptoms in cattle are not found but for herdsmen are presented on (Table 5).

Table 1: Relationship between age group and presence of *M. bovis* status among herdsmen.

Age group (years)	Negative (%)	Positive (%)	Total (%)
15-24	32 (35.2)	0 (0.0)	32 (35.2)
25-34	6 (6.6)	0 (0.0)	6 (6.6)
35-44	43 (47.3)	2 (2.2)	45 (49.5)
45-54	6 (6.6)	1 (1.1)	7 (7.7)
55-64	1 (1.1)	0 (0.0)	1 (1.1)
Total	88(96.7)	3(3.3)	91(100.0)

X^2=4.167 Pvalue = 0.384

Table 2: Distribution of *M. bovis* infection among herdsmen and milk samples in study area.

Table 3: Relationship between ages of rearing of cattle and presence of *M. bovis* among herdsmen in study area.

Age of rearing	Negative	Positive	P value
5-14	26	0	
16-24	10	0	
26-34	32	1	0.390
36-44	18	2	
45-54	2	0	
Settlement			
From cattle to house	67	3	
From cattle to hut	21	0	0.335

Table 4: Relationship between knowledge of *M. bovis* among herdsmen in study area

	No	Yes	Total
Samples	48	43	91
Percentage	52.7	47.3	100
$X^2 = 3.463$		P value = 0.063	

Table 5: Distribution of the presence of clinical symptoms among herdsmen in study area

Symptoms	No	Yes	Total
Fever	50	41	91
Night sweat	85	6	91
Coughing	66	25	91
Weakness	91	91	91

DISCUSSION

Majority of the problems associated with bovine TB are due to the limitation of test diagnosis (Palmer and Waters, 2006; Humblet*et al.*, 2009; Good and Duignan, 2011; More and Good, 2015). In general, sensitivity of official diagnostic test for *M. bovis* infection is low and is affected by several factors such as parasitic infections (Good and Duignan, 2011; More and Good, 2015). Therefore, the need to determine the contribution of bTB to the general tuberculosis burden in a poor resource setting is paramount (Amemor*et al*., 2017).This study evaluates the burden of bovine TB among herdsmen and dairy cattle in Bichi, Kumbotso, Rano, Rogo and Wudil of Kano State in November, 2017 .

The current study found that 3(3.3%) prevalence rate of bTB in herdsmen's sputum to be infected with *M. bovis* found from this study remarkably showed that herdsmen constitute a high risk group and serve as reservoir for *M. bovis*. This prevalence is slightly lower than that of Sydney *et al*., (2014) who obtained a prevalence of 3.6% in Zambia and 3.9% prevalence by Idigbe*et al*., (1986) in Lagos, Nigeria.

The WHO reported in 1998 that 3.1% of TB in humans worldwide is attributable to *M. bovis* and 0.4-10% of sputum isolates from patients in African countries could be *M. bovis*(Michel *et al*., 2010). In Uganda, 7% prevalence rate of *M. bovis* was found by Oloya*et al*., 2008 in humans suffering from cervical lymphadenitis in a pastoral community in Karamojo region (Oloya*et al*., 2008) while in Tanzania, 2001, study conducted of human patients with cases of lymphadenitis reported 16% cases due to *M.bovis* (Kazwala*et al*., 2001). In Zaire, 2 of 5 patients with pulmonary TB were infected with *M. bovis*found from gastric secretions isolated (Mposhy*et al*., 2004). In a study conducted by Sydney *et al*., 20141, using human sputum, based on spoligotyping results, the SB0120/SIT482, lacking spacers 3, 9, 16 and 39-43 in the hybridization pattern, indicates that highly homogenous isolates of *M. bovis* are circulating in both cattle and human district. Also, same spoligotype has been isolated from cattle in Algeria, Brazil, France, South Africa, and Zambia (Haddad *et al*., 2001; Romero *et al*., 2008; Munyeme*et al*., 2009; Sahraovi*et al*., 2009; Parreiras*et al*., 2012) and from humans in

Italy and Germany (Kubica*et al*., 2003; Lari*et al*., 2006; Lari*et al*., 2011). A prevalence of 0% of BTB observed in milk sample of this study was in agreement with the findings of Neeraja*et al*., (2014), who reported 0% in Bangalore, India. Low prevalence of bovine TB in the studied area may be attributed to better farming practices, availability of timely veterinary aid, improved farmer awareness, or treatment of animals with effective herbals to reduce the level of infection against *M. bovis*.

CONCLUSION

This study has documented zoonotic TB in humans in Kano State of Nigeria. Although, a zero percent prevalence of bovine TB was found in this research from raw cow milk,the detection of *M. bovis* antigen from sputum of herdsmen suggests potential of human to human infection especially in dense and over populated area. It also shows that bTB is of public health importance in Africa and World as whole. The study also demands interventions since *M. bovis* infects both human and animal hosts. Hence, its control calls for an intersectoral approach between the Medical, Veterinary professionals and Government. Further studies at molecular level and using a large sample size (raw diary milk from cattle and herdsmen's sputum) in many areas should be tested for *M. bovis* are recommended.

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